2. Scientific Abstract

A new dental caries preventive therapeutic agent, A2JM, is proposed for use in a Phase 1 clinical trial. This agent was created using genetic methods to produce an effector strain of *Streptococcus mutans,* which has both greatly reduced pathogenic potential and increased ability to colonize human teeth as compared to native *S. mutans*. The effector strain, A2JM, has been designed to make substantially less lactic acid than do wild-type strains of *S. mutans*. The lactic acid-deficient *S. mutans* strain was generated by introducing a mutation in a gene involved in the lactic acid synthesis pathway. To prevent the lethality of this mutation to the strain, a recombinant alcohol dehydrogenase gene was introduced into the lactic acid-deficient bacterium to prevent the accumulation of metabolites. The effector strain was constructed using recombinant methods to provide assurance that there were no cryptic mutations and that the construct was genetically stable to the greatest possible extent. The *comE* gene, a gene involved in DNA uptake, was deleted. This deletion should further reduce the possibility for reacquisition of an *ldh* gene by the effector strain and thus reversion to a lactic acid-producing organism.

Increased colonization potential was achieved by starting effector strain construction with a fresh isolate of *S. mutans* strain obtained from a human subject that produces a small, potent, bacteriocin-like inhibitory substance (BLIS). This activity inhibits the growth of all other strains of *S. mutans* against which it was tested.

Finally, a nutritional requirement for D-alanine has been introduced into the effector strain. D-alanine is not naturally present in the environment. The nutritional requirement provides a mechanism that is intended to limit or prevent horizontal transmission of the effector strain from treated to untreated subjects. The preclinical safety data supports a favorable risk/benefit ratio for using A2JM as a replacement therapy for dental caries. The proposed human trial does not expose the subjects to an unreasonable and significant risk of illness or injury, nor does it present the potential for the introduction of a new, more fit and potentially pathogenic organism into the human environment.